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Effects of Oleander Leaves (*Nerium oleander*) against Metabolism, Activity Pattern, and the Leaves Potency as Rice-Field Rat Repellent (*Rattus argentiventer*) †

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Abstract: *Nerium oleander* is a plant that has historically been known as one of the poisonous plants in the world that can be used to control pests. However, studies on the effects of oleander leave against *Rattus argentiventer* as a major agricultural rodent pest are limited. This research aimed to probe the potency of oleander leaves extracted in methanol as rice-field rat repellent. The experiments involve a choice test (T-maze arena) and a no-choice test (metabolic cage) that being analyzed by the T-test using three replications for six days. The result showed that the rats on the T-maze avoided consuming food and beverage near the oleander treatment. The same result occurs in the metabolic cage, which was indicated by the decrease in the average of food and feces, and also the increase in the average of beverage and urine. Besides, the treatment also caused daily activity patterns disorder, which was significantly indicated by the increase of the average percentage of time for resting activities by 22.84% and the decrease of time for locomotion and nesting activities (by 9.71% and 13.13% respectively). Overall, oleander leaves have the potential to provide a repellent effect on rice-field rats, especially in the choice test.

Keywords: extract; plant-based; repellence; *Nerium oleander*; *Rattus argentiventer*; metabolic disorder; apocynaceae

1. Introduction

Rice-field rat (*Rattus argentiventer*) have been classified as important pests in rice cultivation since 1986 [1]. The rate of race-field rat attack on rice plantations in Indonesia averaged 161,000 ha/year, equivalent to the loss of 555 million kg of rice which was enough to consume 6.3 million residents for one year [2]. Synthetic chemical control methods can be used to reduce problems from rat pests. Although it works fast and well, synthetic chemical-based has the following disadvantages such as develop resistance properties, the risk of accidental poisoning of non-target species [3], and also adversely the ecosystem. Alternatively, plant-based repellent is one of a variety of environmentally friendly methods that suitable for pest control, especially rice-field rats whose lives depend on their sense of smell.

Indonesia is a tropical country that has various types of plants that have not been widely used, one of which is *Nerium oleander* [4]. Oleander as widely known as poisonous plants can affect the mortality of the Wistar rat species Rattus norvegicus [5]. In another study has been reported that oleander plants have terpenoid metabolites which can cause a repellent effect on the pest *Ixodes rinicus*; *Plutella xylostella* [6]; *Culex tritaeniorhynchus*, and *Cx. gelidus* [7]. The terpenoid compound study [8], showed this compound has the potential to reject the presence of rats.

An important requirement of repellents is to reject rats by interfering with their sense of smell [9]. Repellent control, if used appropriately in the environment with the target biology, has a high likelihood of being successfully applied to rodents by manipulating their behavior. For instance, in the study of oil of sandalwood, patchouli, and vetiver gave repellent effect to field rice-field rats [10]. However, the potency of oleander plants as plant-derived rice-field rats repellents are still unknown. Therefore, this study is one of the promising male and female rice-field rat control tools is the application of plant secondary metabolite (PSM) odor mixtures by extract of oleander leaves that can be seen from its effect on metabolism, daily activity patterns, and the potency as a repellent.

2. Experiments

2.1. Study Site

The experiments were conducted at Laboratory of Pests, Division of Pests Vertebrate, Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Padjadjaran (6°35′32.72″ S & 107°64′55.73″ E) and Laboratory of Rats, Indonesian Center for Rice Research (6°55′33.0″ S & 107°46′24.6″ E), from February until June 2019.

2.2. Rice-Field Rats

Rice-field rat originated from Sukamandi rice-field, Indonesian Center for Rice Research that being collected by implementing the LTBS (Linear Trap Barrier System). The 24 rice-field rats used as test animals were selected based on health conditions, sex (12 mature males and 12 not pregnant females), and body weight (weight range between 90–130 g). The collected rats were adapted to food (brown rice) and beverage (tap water) for one week in aluminum-framed cages.

2.3. Plant Materials and Extraction

The young oleander leaves collected along the *irigasi* street, Bandung, West Java, Indonesia. The collected leaves were washed with tap water and being air-dried for about 2 weeks, and then blended into a powder. Leaves then finely soaked with methanol (75%) with a ratio of ingredients and solvents (100 g:1 L) over 4 h. The sample was then filtered with Whatman 2 mm filter paper, and distilled using automatic steam distillation (±80 °C) for about 1.5–2 h. The 500 mL extract sample was then transferred into bottle spray for the treatment.

2.4. Choice Test in T-Maze

Choice test research on the repellent of oleander leaves on rice-field rats in the T-maze (Figure 1) at the Rat Laboratory, Indonesian Center for Rice Research. The rat first entered from the start pipe then chooses to eat and drink between the two pipe arms which are left hand (a) was a treatment room with extract of oleander leaves and right hand (b) was a control room without extract of oleander leaves. The extract was sprayed onto two cloth sheets (10 × 10 cm) with a hand sprayer (5 mL/sheet) and placed inside room treatment (a) that close to food and beverage containers. Rice-field rat was individually kept in the T-maze arena, provided with food (brown rice 10% of body weight) and beverage (100 mL of water), at the same amount both in the left and right hand, every day in the afternoon for six days experiment. The observations were carried out every day in the morning, including food consumption (g), water consumption (ml).

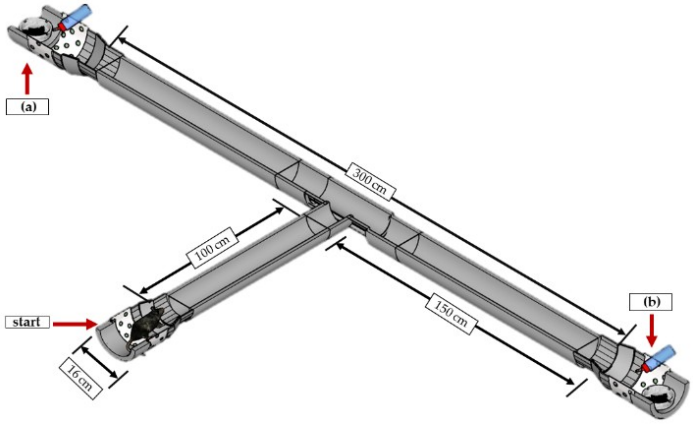

Figure 1. T-maze arena prototype and pipe size. (**a**) extract of oleander leaves treatment and (**b**) control room without extract of oleander leaves.

2.6. No-Choice Test in Metabolic Cages

No-choice test research on the effect of oleander leaves on metabolic parameters was carried out at the Pest Laboratory, Faculty of Agriculture, Universitas Padjadjaran. The extract was sprayed onto two cloth sheets (10 × 10 cm) with a hand sprayer (5 mL/sheet) and placed inside room treatment that close to food and beverage containers (Figure 2). Rice-field rat was individually kept in the metabolic cage, provided with food (brown rice 10% of body weight) and beverage (100 mL of water), every day in the afternoon for six days experiment. The observations were carried out every day in the morning, included food consumption (g), water consumption (ml), feces production (g), urine production (ml). Furthermore, the test was carried out using CCTV (Closed Circuit Television) in metabolic cages for the observed daily activity of the rice-field rats. The parameters include time spent on movement activities (locomo-tion), eating and drinking activities (foraging), and resting and sleeping activities (resting). The activities are viewed every minute in 12 h (18.00–06.00) every day, the results being added up and divided by six (days of observation), then converted into percentages.

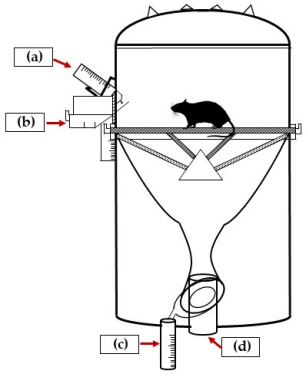

Figure 2. Metabolic cage prototype. Container of (**a**) beverage; (**b**) food; (**c**) urine; (**d**) feces.

2.7. Data Processing and Analysis

The treatment was done with three replications for both female and male rice-field rats. The experimental data compared using the average difference test method with two independent data (Independent Sample T-test) between extract and control treatment. Statistical results and the significance assessed at 95% confidence level to compare the differences between treatment means that analyzed using Statistical Package for the Social Sciences (SPSS) software version 25.0 in Windows.

3. Results

3.1. Choice Test in T-Maze Arena

The result below showed that the average of oleander leaves repellency on the choice tests on food and drink consumption each day of rice-field rats in the T-maze after six days of the observations (Table 1). The rice-field rats tended to eat in the control room (B) rather than treatment room (A), evidenced by the amount consumed for female rats differences significantly by 3.42 g and male by 2.88 g. It is also noticeable that the rice-field rats also drink more in the control room rather than in the extract room that shown differences significantly for females 4.55 mL and male by 5.55 mL.

Table 1. The average amount of food and drink consumption between female and male rice-field rats each day after six days of the choice test in the T-maze.

Sex	Treatment	Average Per Day	
		Food Consumed (g)	Drink Consumed (ml)
Female	Extract	2.44 *	4.56 *
	Control	5.86	9.11
Male	Extract	3.29 *	4.56 **
	Control	6.17	10.11

Symbol asterisk (*) indicates a significant difference between the treatments. * $p < 0.05$; ** $p < 0.001$ highly significant.

3.2. No-Choice Test in Metabolic Cage

3.2.1. The Effect of Oleander Leaves on Rice-Field Rats Metabolism

Based on Table 2, both male and female rice-field rats tended to consume highly significant food and drink in the metabolic cage without extract of oleander leaves (control). On the other hand, a small amount was found when the rice-field rats were exposed to the metabolic cages that contained the extract of oleander leaves. In comparison, the difference number between the extract treatment and control on the average amount of food and drink consumption on females 3.59 g and 6.34 mL, whereas the male is 2.94 g and 3.73 mL. It can be clearly seen that the metabolic disorder effects of the extract of oleander leaves were also found in feces and urine production of male and female rice-field rats in the metabolic cage (Tabel 2). The study in the metabolic cage without methanol extract of frangipani leaves showed that both male and female rice-field rats produced a significant difference number of feces and urine which is related to the amount of food and drink the rats previously consumed.

Table 2. The average amount of food, drink consumption, feces, and urine between female and male rice-field rats each day after six days of the choice test in the metabolic cage.

Sex	Treatment	Average per day			
		Food Consumed (g)	Drink consumEd (mL)	Feces (g)	Urine (mL)
Female	Extract	6.13 *	4.44 **	0.52 **	1.78 *
	Control	9.72	10.78	1.56	3.5
Male	Extract	7.08 *	8.00*	1.05	3.33
	Control	10.02	11.72	1.75	4.11

Symbol asterisk (*) indicates a significant difference between the treatments. * $p < 0.05$; ** $p < 0.001$ highly significant.

3.2.2. The Effect of Oleander Leaves on Rice-Field Rats Daily Acticity

The bar graph below depicts the percentages of time spent on rice-field rats' daily activity for 12 h in a metabolic cage (Figure 3). It is found that both female and male rice-field rats tend to do more resting activities (female 55.8% and male 40.9%) when in the metabolic cage that have the extract of oleander leaves in it. Due to extract treatment, locomotion and foraging activities percentages significantly less than the control. However, the rice-field rats in control treatment mostly spend their time on foraging then followed by locomotion activities. One and the other sexual category of rice-field rats tend to not resting on the observation time (18.00–06.00).

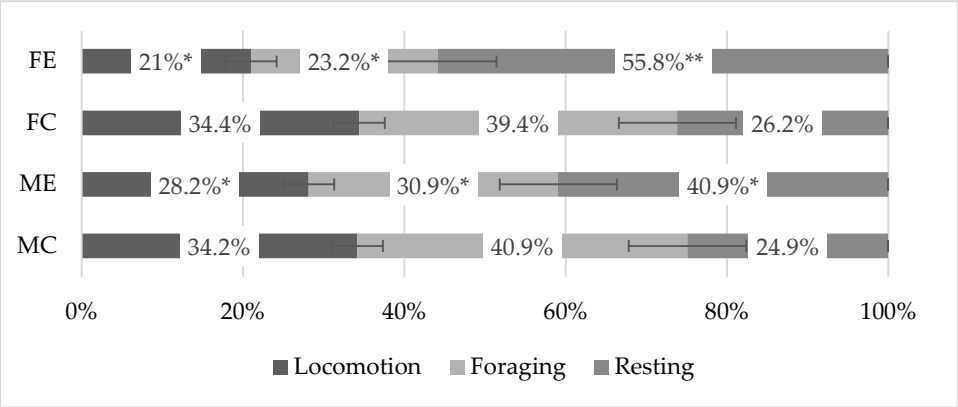

Figure 3. Comparison of the percentage averages daily activity pattern in 12 h (18.00–06.00) using CCTV every day until six days of observation in the metabolic cage. Female Extract (FE); Female Control (FC); Male Extract (ME); Male Control (MC). *Extract* implies the rat is in the metabolic cage with extract of oleander leaves treatment, whereas *control* implies without any treatment in the metabolic cage. The standard error (SE) of the mean is showed by a vertical bar. The symbol asterisk (*) indicates a significant difference between the treatments. * $p < 0.05$; ** $p < 0.001$ highly significant.

4. Discussion

Rice-field rats are animals that have advantages in the sense of smell that is perfectly developed [11] that usually being used to identify prey, avoid danger, and search for food [12]. The experiment showing that the rice-field rats tend to eat and drink in the control room compared that those placed near oleander leaves treatment of the choice-test in T-maze arena. This phenomenon has occurred because the rats have two options to choose food and drink in the most pleasant-smell-like control room rather than the uncomfortable-smell in the oleander leaves treatment room. During six days of research, because the rats already know which safe place they can go, the tendency to go to the same place is getting bigger. The results of the observations made are supported by research results [13] showed that the rats' brains are perfectly developed so they can learn and remember something well.

The consistent result also showed from the no-choice test in the metabolic cage. Even though the amount of food and drink were more than in the choice test, this situation can be considered reasonable because rats have no choice over food and drink they consumed. However, the amount consumed of the rats in cages with oleander leaves treatment was significantly different from the control. Food and drink is one of the most important sources of energy in the growth and development of rats. The circumstances of this experiment are thought to be due to neophobic behavior that caused stress actions. Rats that experience stress eats less food than rats not stressed [14].

Stress conditions that can determine the energy that is logged, so that it can reduce food motivation and intake. The behavior was shown by rats due to the influence of oleander leaves repellent at a dose of 5 mL which was applied. A substance can only be considered a repellent when it causes an organism to orient its motion away from its source [15]. Less eating behavior in the

treatment cage is thought to be influenced by stress reactions that orient the rats to stay away because of the unusual and uncomfortable smell from oleander leaves.

Food intake is related to feces production. Based on the observations, rice-field rats that were in oleander leaves treatment conditions that had a lower average number of feces than controls. Low urine production in rice field rats also may cause due to stress from the aroma of oleander leaves, related to stress-related displacement associated with sympathetic nerves that work more actively with the help of bladder contractions [16]. This phenomenon is considered fair from the research results because the smaller food and drink rats consumed, then the smaller amount of feces and urine they will produce. The effects may also have occurred.

Most of the normal activities are carried out by rice-field rats starting from dusk until before dawn. This is because rats are one of the nocturnal or active nocturnal mammals. The research was held at 18.00–06.00 to see if the behavior of the rats changed because of the oleander leaves treatment. The study showed that the rice-field both of female and male rats in control cage tend to spend their time normally to do foraging and locomotion activities. In contrast, rats do more sleep or rest activities rather than doing other activities (foraging or locomotion) that is thought to originate from the influence of the repellent. The aroma arising from oleander leaves is thought to provide uncomfortable conditions for rice-field in metabolic cages so that the activity of rats is disrupted. The defensive behavior is carried out by rats when under stress or uncomfortable conditions [17].

In addition, both of the tests showed that male rice-field rats tend to consume more food and drink compared to female rats. This is supported according to research [18] showed that male rats exhibit a strong determination to survive so they are more risk-taking than female rats. Biologically, female rats tend to be more sensitive to new things thus it is suspected that the ability to adapt to the female rat is longer than the male rat [19]. Females tend to reduce their activity more than male when under stress [20]. Thus, the stress felt by the rats due to the influence of oleander leaves results in significant differences between treatment and control. As a result, gender differentiation can also make a difference in the measured patterns of rice-field rats activities.

5. Conclusions

This study concludes that extract oleander leaves gives a repellent, metabolic, and daily activity pattern disorder to both female and male rice-field rats. The rats tend to reduce their will to consume food or water that affects the amount of urine and feces they produce. Moreover, oleander leaves also affect the rice-field rats' daily activity patterns which showed by a change in their habitual on the observation time (18.00–06.00) from actively foraging or locomotion to become passively (resting). It is also found that male rice-field rats tend to take more risk to consume more food and drink rather than female rats. Owing to the experiment that has been done, it is worth investigating more further regarding the isolation of bioactive compounds of oleander leaves as a repellent against rice-field rats or makes other experiments on other species.

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Conflicts of Interest: The authors declare no conflict of interest.

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